Histopathological Effects On Liver And Ovary Of The Fish, *Clarias batrachus* Exposed To Sub- Lethal Concentration Of Chlorpyrifos 50% + Cypermethrin 5% EC

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ABSTRACT

The intention of present study is to evaluate the toxic effects of a hybrid pesticide (i.e. combination of two classes of pesticides), Chlorpyrifos 50% + Cypermethrin 5% EC (Organophosphate + Synthetic Pyrethroid) on the two vital organs i.e. liver and ovary of an air breathing fish, *Clarias batrachus* exposed to the sub- lethal concentration. The study revealed histopathological changes in the liver which include irregular hepatocytes, nuclear hypertrophy, cytoplasmic vacuolation, glycogen depletion etc. Histopathological changes observed in the ovary included oocyte shrinkage, oocyte degeneration, destruction of ovigerous lamellae, cytoplasmic necrosis etc. These observations are thus indicative of the toxic effects caused by this hybrid pesticide at cellular/histological level in the organs of the fish, *Clarias batrachus.*

Keywords : Pesticide, Organophosphate, Synthetic Pyrethroid, *Clarias batrachus,* Liver, Ovary, Hepatocytes, Oocytes.

Pages: 6 References: 24

INTRODUCTION

Pesticides have been used in agriculture for decades to enhance food production by eradicating out disease causing insects and vectors (Prakasam *et.al.,* 2001). These chemicals may reach into the environment such as lakes and rivers through rains, winds or mishandling, thus affecting many non- targeted organisms. The injuries caused by the pesticides are incontestable. Their significant increase in water resources has led to the deleterious effects on many aquatic organisms (Livingstone, 2001; Matsumoto *et.al.,* 2006). Fish are able to uptake and retain different xenobiotics dissolved in water via active or passive processes. They can be used to detect and monitor pollutants released into their aquatic environments. Sub- lethal concentrations of pesticides in aquatic environment cause structural and functional changes in the body of affected fish (Sancho *et.al.,* 2003). Histological analysis provides unique perspective linking the injudicious use of harmful chemicals and pesticides and their unwanted results in the body of the non- target organisms. Chronic exposure of sub-lethal concentration of pesticides has been found to cause moderate to severe histopathological changes in the tissues and organs of fish. Recent trend in the increased use of organophosphates (OPs) and synthetic pyrethroids in agricultural practices is a ma-

jor issue of concern. Exposure of aquatic ecosystem to these insecticides is difficult to assess because of their short persistence in water column due to low solubility and rapid degradation. However, monitoring of these insecticides is important because of their highly toxic nature. All the organophosphates are potent nerve inhibitors. They block the active sites of the enzyme acetylcholinesterase (AChE) that breaks down and hydrolyses the neurotransmitter acetylcholine (Ach) from the nerve synapse. Chlorpyrifos is a synthetic organophosphate (OP), non- systemic and broad spectrum insecticide, acting as a cholinesterase inhibitor and may get assess into the body via dermal contact, ingestion and respiratory pathway. Similarly, synthetic pyrethroids are also widely used in agricultural practices. Pyrethroids are several orders of magnitude more toxic to fish than the organophosphate pesticides (Oros and Werner, 2005). They are potent neurotoxicants that interfere with nerve cell functions by interacting with voltage- dependent sodium channel, resulting in repetitive firing of neurons and eventually causing paralysis (Shafer and Meyer, 2004). Present investigation reveals the combined toxicity of both the classes of pesticides i.e. organophosphate and synthetic pyrethroids on the liver and ovary of the fish, *Clarias batrachus* (Family Claridae). *Clarias batrachus* also known as 'Magur' in North Bihar is a commercially important fish. Literature review reveals that little work has been done with the fish in the field of pesticide toxicology.

MATERIALS AND METHODS

Procurement of the test animal

Healthy and living specimens of *Clarias batrachus* (Linn.) of size roughly in the range of 15- 25 cm. and weight 150- 220 gm were collected from a local pond in Muzaffarpur, Bihar. These were disinfected by subjecting them to a bath of 0.1% aqueous potassium permanganate ($KMnO_4$) solution for 15 minutes to remove any dermal infection. The fish were now transferred to a large tank containing water and kept for 20 days for acclimatization. During the period of acclimatization they were fed alternatively with pieces of soyabean and chick's intestine. The average physico- chemical conditions were maintained optimum during this period. The water of the tank was renewed every two days to minimize contamination as well as maintaining the average physico- chemical characteristics of the water.

Bioassay test

An acute toxicity LC_{50} test by the static renewal bioassay method was conducted to determine the toxicity of Chlorpyrifos 50% + Cypermethrin 5% EC in the fresh water fish, *Clarias batrachus* exposed to various concentrations of the pesticide till 96 hrs. Based on the mortality observed at different concentrations during 96 hrs, LC_{50} value was estimated for different periods such as 24 hrs, 48 hrs, 72 hrs and 96 hrs using straight line graphical interpolation method. For exposing the test animal to sub- lethal concentration of the pesticide, 1/12th of the 96 hrs LC_{50} value (i.e. 0.5 μL/Litre of water) was taken and fish were exposed to this concentration for 30 days. After this period, the fish were sacrificed and their organs extracted. These organs were then fixed in fixatives (formalin and Bouin's solution aqueous). The tissues were then dehydrated, cleansed and embedded in wax. Thin sections were then cut with microtome and observed under microscope after undergoing standard staining protocol (H&E Staining).

OBSERVATIONS

Physico-chemical characteristics of the test water

The results of the physical and chemical analysis of the test water estimated by using procedures as mentioned by APHA (2005) are given in given table.

Table 1: Average physico- chemical characteristics of test water

1.1	Temperature	26° ± 2.0 °C
1.2	pH	7.12 ± 0.14
1.3	Dissolved O_2	7.42 ± 1.10 ppm
1.4	Total hardness as $CaCO_3$	164.76 ± 5.38 ppm
1.5	Total alkalinity as $CaCO_3$	148.64 ± 7.77 ppm
1.6	Chlorides	14.42 ± 1.05 ppm

HISTOPATHOLOGICAL STUDIES OF LIVER

Histology of the normal liver

The liver shows homogenous mass of polyhedral cells or hepatocytes arranged in groups (Fig.1&2). Each hepatocyte contains a centrally located spheri-

cal nucleus with prominent nucleolous. The cytoplasm is granular taking deep basophilic stain. These hepatocytes are radially arranged around a central vein in interconnecting lamina (Fig.1&2). The hepatic triads consist of hepatic veins, hepatic artery and bile duct.

Histology of the liver exposed to the pesticide

The main alterations found in the liver were altered tissue architecture (Fig.4), irregular shaped nuclei placed laterally (Fig.3), nuclear hypertrophy, nuclear vacuolation and presence of melanomacrophage aggregates (Fig.3). Cytoplasmic and nuclear degenerations were also observed (Fig.5). The tissues were slightly to moderately damaged as is evident by altered histological structure and cytoplasmic degeneration (Fig.4&5). Bile stagnation was also identified as brown- yellowish granules in the cytoplasm. The liver showed vacuolated hepatocytes and frequent necrosis (Fig.3).

HISTOPATHOLOGICAL STUDIES OF OVARY

Histology of the normal ovary

Each of the two ovaries is covered externally by a thin peritoneum followed by tunica albuginia composed of the connective tissue muscle fibres and blood capillaries and the innermost layer of germinal epithelium consist of single layer of cuboidal cells. The ovary shows various stages of oogenesis with developing oocyte follicles and oocytes in different developmental stages (Fig.6&7). Graffian follicles (Fig.7) as well as Corpus luteum (Fig.6) were also seen.

Histology of the ovary exposed to the pesticide

Marked alterations were observed in the histological structure of ovary exposed to the pesticide. Oocytes growth was greatly retarded with absence of stage III & IV oocytes and increased atretic oocytes (Fig.8). Oocyte shrinkage was quite evident. Cytoplasmic retraction and cytoplasmic degeneration were also observed along with clumping of oocytes (Fig.10). Destruction of the follicular epithelium (Fig.9), increased intrafollicular space, vacuolated cytoplasm and necrosis in the cytoplasm were seen. Pesticide exposure also disrupted the normal rhythm of oocyte maturation and differentiation.

Fig. 1

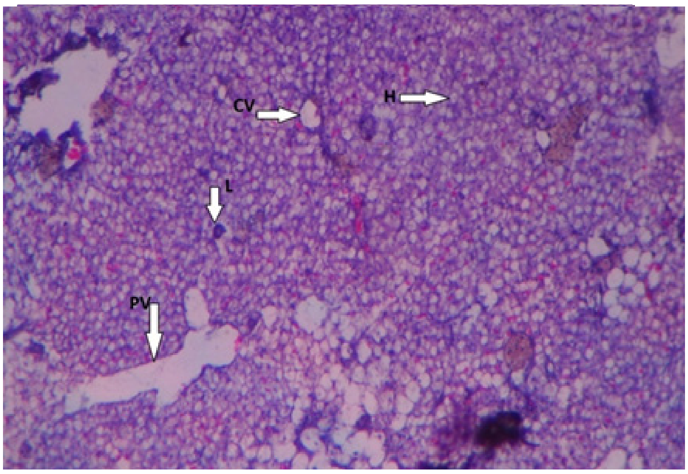

Fig. 2

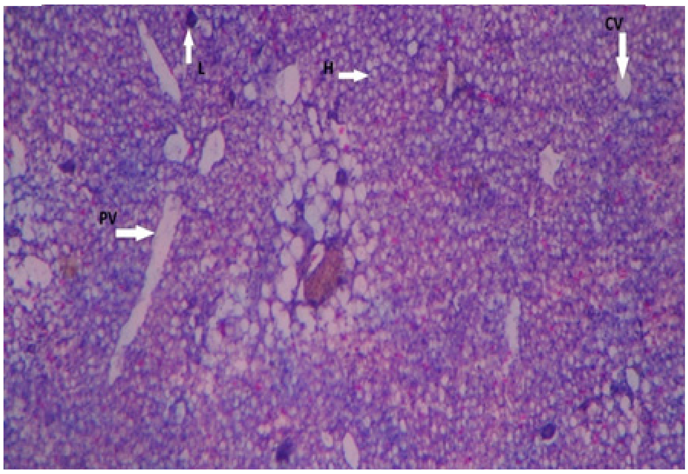

Fig. 1 & 2. Histological slides of normal liver showing normal histological architecture like central vein (CV), portal vein (PV), hepatocyte (H) and lymphatic nodule (L)

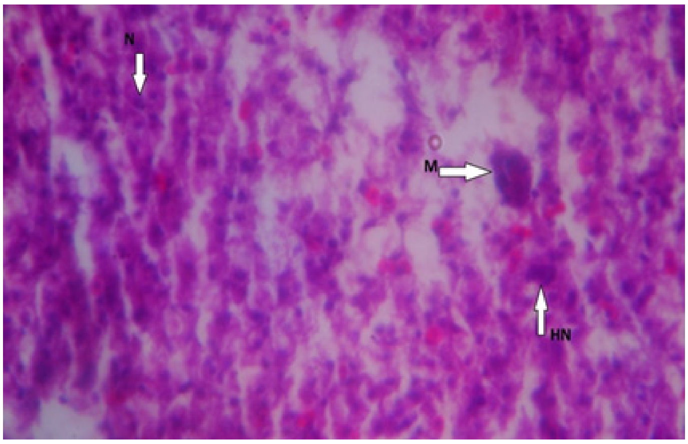

Fig.3. Histological slide of treated liver showing hepatic necrosis (HN), melanomacrophage aggregate (M) and nucleus in lateral position (N).

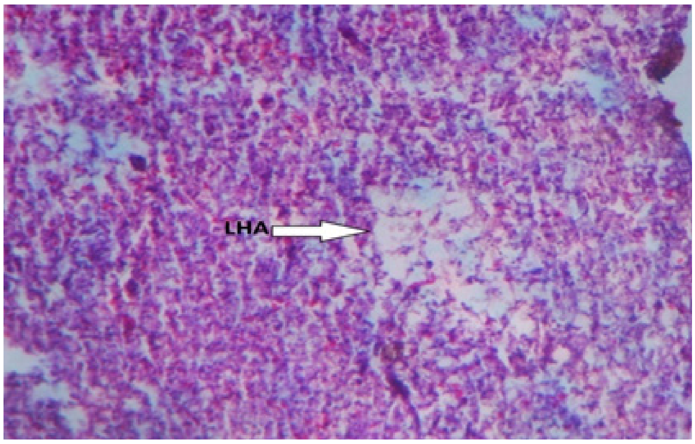

Fig.4. Histological slide of treated liver showing altered hepatic architecture (LHA).

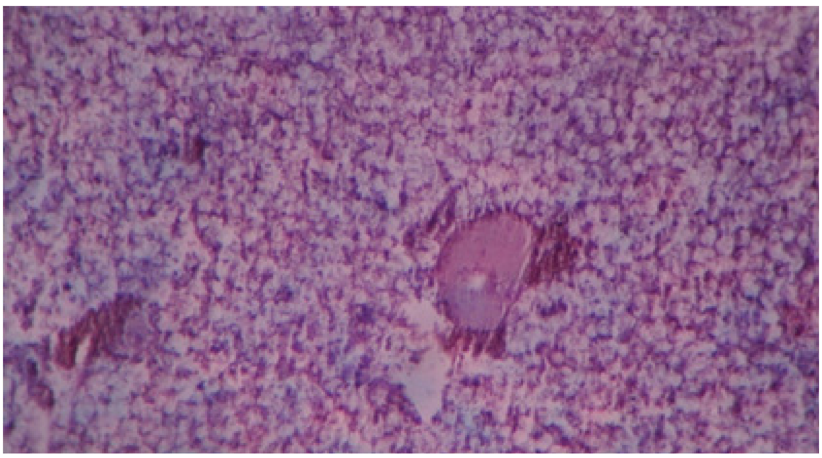

Fig.5. Histological slide of treated liver showing cytoplasmic degeneration (less intense eosin stain).

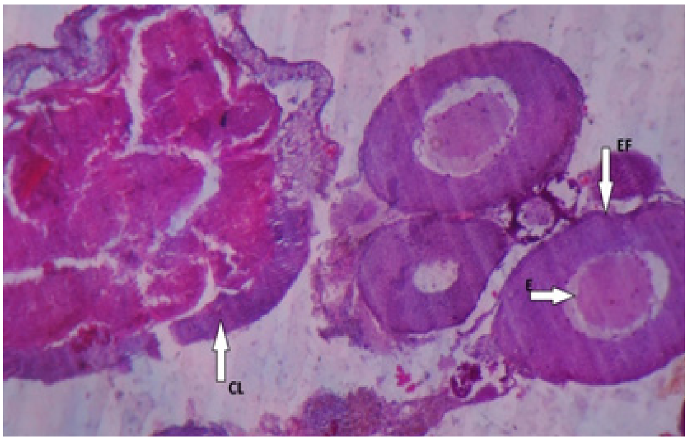

Fig.6. Histological slide of normal ovary showing oocyte follicle (EF), egg (E) and corpus luteum(CL)

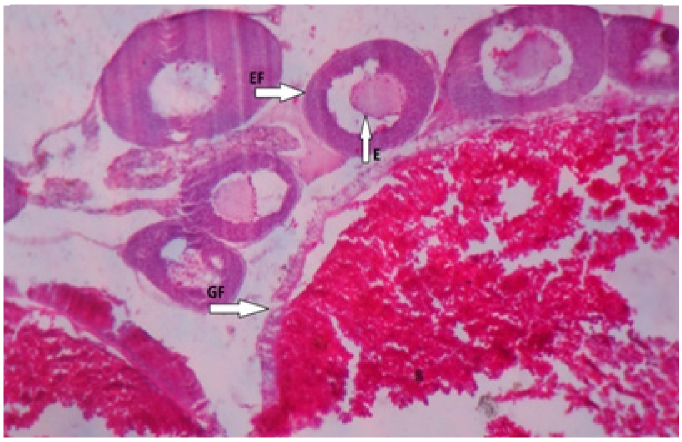

Fig. 7. Histological slides of normal ovary showing normal developing oocyte follicle (EF), egg or ovum (E) and Graffian follicle (GF).

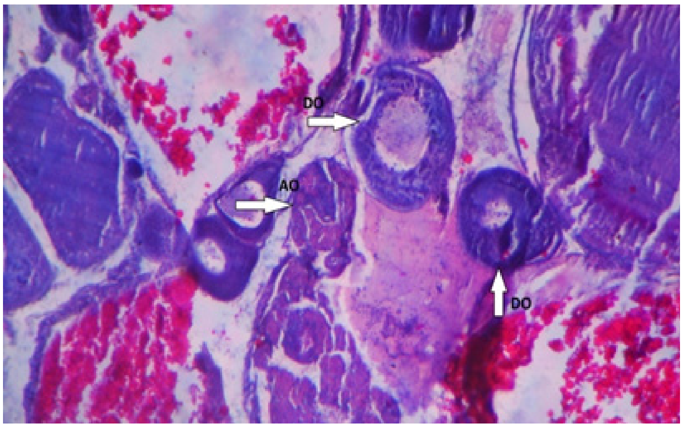

Fig.8. Histological slide of treated ovary showing atretic oocyte (AO) and degenerating follicle (DO)

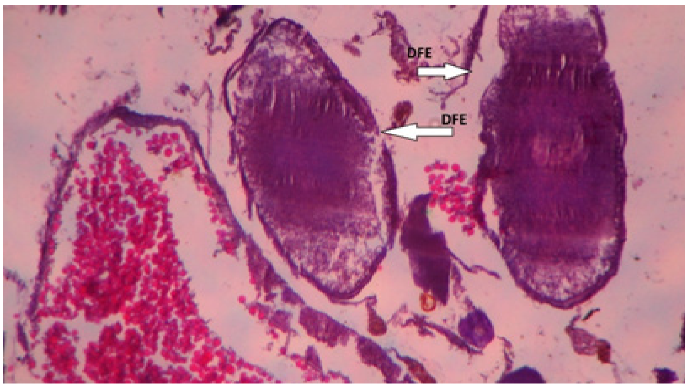

Fig.9. Histological slide of treated ovary showing degenerated follicular epithelium (DFE).

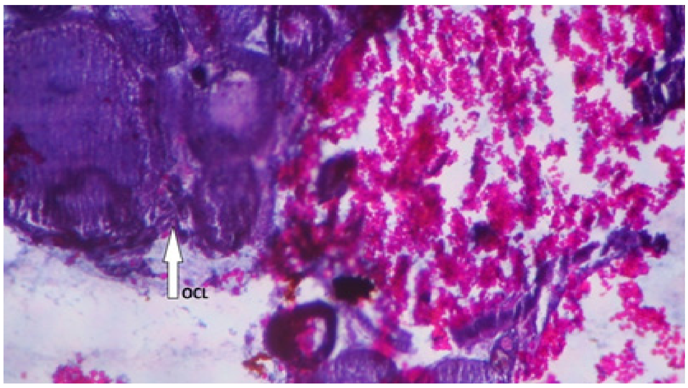

Fig.10. Histological slide of treated ovary showing clumping of oocyte follicles (OCL).

DISCUSSION

Histopathology Of Liver

The toxicity of pesticides is dependent on the physical and chemical characteristics of water. Therefore, the physical and chemical analysis of the test water becomes essential before performing the experiment. In the present study, marked histopathological alterations were observed in the liver and ovary of the fish exposed to pesticide. Liver is the most important organ associated with the detoxification and biotransformation process. Due to its function, position and blood supply (Van der Oost *et.al.,*2003), it is also one of the organ most affected by contaminants in the water (Rodrigues & Fanta, 1998). Irregular shaped hepatocytes, cytoplasmic vacuolation, nucleus in lateral positions were some of the alterations observed during the experiment. Cytoplasmic and nuclear

degeneration were common. Such anomalies (irregular shaped hepatocytes, cytoplasmic vacuolation etc.) were also described in the siluriform Corydoras paleatus contaminated with organophosphate pesticides (Fanta *et.al.,* 2003). Vacuoles in the cytoplasm of the hepatocytes may contain lipid and glycogen. Glycogen depletion in the hepatocytes of the stressed animals has been reported by workers like Hinton & Lauren (1990), Wilhelm Filho *et.al.,* (2001). Glycogen depletion in hepatocytes may be linked with the fact that glycogen acts as a reserve of glucose to supply higher energy demand under stressed conditions. Often, quantitative alterations in the hepatic morphology are visible macroscopically as changes in liver size and coloration; and histologically as variations in cellular architecture, hepatocellular vacuolation and altered staining characteristics (Wolf and Wolf, 2005). Increased hepatocellular vacuolation is more commonly associated with toxic conditions (Wolf and Wolf, 2005). For example, cloudy swelling and hydropic degeneration were reported in Nile tilapia exposed to sub- lethal concentration of ammo-

Histopathology Of Ovary

The ovary of the fish was a paired, elongated and tubular structure fully laden with eggs especially during the breeding season. Severe histopathological changes were observed in the histological slides of the ovary exposed to the sub- lethal concentration of the pesticide. Oocyte shrinkage along with retarded oocyte growth was quite obvious. Absence of stage III and especially stage IV oocyte was noticed. Reduction in the ovarian weight as well as retarded growth of the pre- vitellogenic oocytes have been also reported in fish, *Glossogobins giuris* (Ham.) exposed to lower dosage of malathion (Ramachandra M. M. 2000). A higher dose resulted in the degeneration of the immature oocytes and rupture of follicular epithelium. Present findings also reveals similar degenerative changes and are thus in accordance with the previous findings. Degeneration of the follicular walls, connective tissues and vacuolation in the ooplasm was also apparent. Dutta *et.al.,*(1994) has reported microscopic changes in ovigerous lamellae, clumping of

CONCLUSION

Present investigations have thus revealed the degenerative effects of the pesticide, Chlorpyrifos 50% + Cypermethrin 5% EC on the liver and ovary of the fish. *Clarias batrachus* exposed to the sub- lethal concentration of the pesticide. Histopathological changes in the liver include altered hepatic tissue architecture, irregular nuclei, nucleus in lateral position, cytoplasmic vacuolation, presence of melanomacrophage aggregates and bile stagnation. Since liver is the most important organ of the body involved in a variety of metabolic functions, degenerative changes in its structure may prove disadvantageous to the life of the fish. Similarly, changes observed in the ovary exposed to the sub- lethal concentration of the pesticide include retarded oocyte growth, presence of atretic oocytes, cytoplasmic retraction and degeneration, destruction of the follicular epithelium and clumping of oocytes. These changes in the ovary of the fish are not good from the point of view of normal reproductive process. Since ovary is an important organ involved in the activity of reproduction as well as hormone secretion, changes in its structure and function may result in decreased egg production and hormonal imbalance. These changes may lead to reduction in fish population as well as alteration of the normal physiology in general and reproductive physiology in particular of the fish. Our findings

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